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EAGER: Collaborative Research: Developing Transformation Technologies for Porphyra

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Title:
EAGER: Collaborative Research: Developing Transformation Technologies for Porphyra

Project Participants

Senior Personnel

Name: Brawley, Susan
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Brodie, Juliet
Worked for more than 160 Hours: No
Contribution to Project:
New collaboration to obtain information on young blade germination planes from conchospore-generated blades in NE Atlantic.

Post-doc

Name: Miranda, Lilibeth
Worked for more than 160 Hours: Yes
Contribution to Project:

Graduate Student

Undergraduate Student

Name: Manning, Joshua
Worked for more than 160 Hours: Yes
Contribution to Project:
This person helps maintain cultures and does general lab maintenance including weekly trips to coast for seawater. He also helps collect material of P. umbilicalis in the field when needed for the transformation studies.

Name: Rankin, Joseph
Worked for more than 160 Hours: Yes
Contribution to Project:
Mr. Rankin did lab clean-up (dishes etc.) and seawater runs last year. This year, he is doing his senior capstone thesis on the bacteria remaining in the blade of P.um.1 and assisting in collection of NS for electroporation experiments.

Technician, Programmer

Other Participant

Research Experience for Undergraduates

Organizational Partners

Other Collaborators or Contacts

Personnel from the Joint Genome Institute have provided the project with promoter information from the preliminary draft of the P. umbilicalis genome.

Activities and Findings

Research and Education Activities:

The major goal of this project was to develop transformation technology for the red marine alga Porphyra umbilicalis, which is the subject of a JGI sequencing project. To use experimental biology to exploit the genomic sequence of this organism in metabolic and developmental studies, transformation is important. Several groups in Japan, China and the US have published research since 1994 on attempts to transform Porphyra; in all of these attempts, transformation was unstable and occurred at low efficiency. Briefly, I describe the technical approach we used. Although this also proved unsuccessful, the likely bacterial interference in the screening assay, and related difficulties in the genome project, led us to determine more about the bacterial community associated with Porphyra blades (natural, and the clonal lab blades used for the genome project). This information provides the first pyrosequencing study of a marine macrophyte?s bacterial community and has led to novel understanding of bacterial-algal symbiosis (Miranda et al., in revision). We also explored differential regeneration of the blade during acute low salinity stress. In addition to the personnel trained during the grant, Brawley developed a 3 week research project for her SMS Marine and Freshwater Algae class, which culminated in a service learning project in spring 2012 for a Maine company that harvests Porphyra.

Findings:

Transformation research

Protocols for transformation of neutral spores were developed by electroporation, lithium acetate, and glass beads. By PCR, we obtained a resistance gene from the dpGreenKanT vector that permitted us to screen for successful transformation with geneticin antibiotic. Native promoters, found upstream of the carbonic anhydrase or glyceraldehyde phosphate dehydrogenase genes in the JGI sequencing project?s archive, were used to drive expression in a construct that was ligated and cloned in the pGEM T easy vector. Neutral spores (NS) obtained from the edge of mature P.um.1 clonal blades in the lab were treated with agarase (5U) to remove mucilage that adheres unevenly to their surfaces upon release. Electroporation of the plasmid into neutral spores was optimized (125 μF capacitance, 0.4 kV, 100 ohms resistance and pulse duration of 4.8-5.1 ms; Biorad Gene Pulser) to provide a germination level of 50% against controls that were not elecrtroporated, which germinated at 97-100%. Screening of appropriate levels of geneticin to use as a screening agent to detect successful transformation was repeated at the beginning of the project period with 400 μg/mL proved successful in preventing neutral spore germination. Attempts to introduce the plasmid into NS with lithium acetate and glass beads followed standard protocols developed for Chlamydomonas. Neutral spores did not develop in geneticin when all controls were killed by it; this led to our failure to date to develop stable transformation for Porphyra. Concern that the geneticin might be killing bacteria required by NS for development led to our investigation of bacteria associated with Porphyra blades. This information may help to develop transformation in the future. Understanding the bacterial community and how to remove it has proved helpful toward obtaining the P. umbilicalis nuclear genome.

Bacterial Community

We obtained 147,880 high quality pyrosequences from the V8 and V5-V6 regions of the 16S rDNA in order to determine what bacteria remained in the clonal lab blades being used for the genome project and those remaining attached to neutral spores in the transformation

work. We compared the lab blade communities to the natural bacterial metagenomc on 5 blades collected in fall and 5 collected in winter. Only 1 OTU (a Saprospiraceae, Bacteroidetes) was universally present on the 2 lab blades and 10 field blades, out of a total of 2,526 OTUs (0.03 distance) formed from sequences within the V8 library. A similar number of OTUs was identified in the V5-V6 library, but no bacterium was universally present on blades in the V5-V6 library. A number of OTUs attained abundance of 1% or higher on one or more blades. Further, we identified a core microbiome that was shared among blades in fall, winter and the lab that consisted of 13 OTUs (V8) The clonal P.um.1 lab blades were characterized by a very high abundance of Flavobacteria (about 75%) and a smaller number of Planctomycetes (about 4%) and Proteobacteria (especially Sphingopyxis). The proportions of Flavobacteria and Sphingobacteria in the Bacteroidetes on Porphyra blades were nearly inverted on lab and field blades, respectively. Lewinella was the most abundant genus on all field blades but it was comprised of 66 OTUs, and only a few of these were among the most abundant OTUs. Many of the bacteria remaining of Porphyra blades are reported to be geneticin sensitive, and they provide specific, critical morphogens for Porphyra germination and development. The striking abundance of cell-wall digesting bacteria on Porphyra, including several bacteria known to produce morphogens that maintain multicellularity in green algae such as Ulva led us to propose a new model for the symbiosis between some bacteria and macroalgae (Miranda et al., in revision).

Planctomycetes

Special attention was also given in the EAGER research to the planctomycetes, because they proved to be major, troublesome contaminants in the genomic sequencing effort at JGI. Isolations of pure cultures of planctomycetes from blades resulted (so far) in one pure culture, and others are being screened. In collaboration with scientists at JGI, 3 new planctomycete genomes have been assembled and a manuscript is in preparation on the carbohydrate digesting capacity of these bacteria and other biological features discovered in their genomes (Kim et al., in prep).

Stress tolerance

An area of particular interest, as proposed in our EAGER application, is to understand the high stress tolerance of P. umbilicalis, which is an intertidal alga. We investigated tolerance to acute low salinity (4 psu), and found that regenerative ability of cells in the thallus varies, with a gradient in recovery of cells from the rhizoid (low) to the tip of the blade (high). Callus formation is one product of this treatment, as is endospore formation from recovering cells, which form islands in the treated blades. In nature, such acute freshwater stress would lead to millions of propagules being released from each stressed blade. P. umbilicalis recolonized the NW Atlantic from the NE Atlantic after the last glaciation or persisted in small refugia off the NW coast of North America; the Brawley lab has earlier published on the variation in life history of Porphyra umbilicalis across its Altantic range (asexual blades that reproduce only by neutral spores in the NW Atlantic; a sexual heteromorphic life history in the NE Atlantic). It is likely that the regenerative ability we have observed was important in the survival and recolonization of the asexual strain and our new findings add to the value of P. umbilicalis as a model for stress tolerance (Rankin et al., 2012).

Training and Development:

Dr. Miranda received her Ph.D. at Univ. Connecticut and worked for a year for a biofuels' project (postdoc) at Texas A & M. She had no prior experience with macrophyte culture, and is now expert. She also had little experience with microscopy and has learned fluorescence microscopy. Her molecular background is strengthened by preparation of the constructs for transformation and by associated qPCR studies that led to our choice of promoters. Dr. Miranda spent several weeks in Dr. Grossman's laboratory working on the constructs (He has an associated part of this EAGER Collaborative Research Grant under a different grant

number).

Two undergraduates (Josh Manning and Joseph Rankin) participated in this research, including completion of Rankin's senior capstone thesis work in spring 2012. These undergraduates presented abstracts of their research in 1 (Manning) and 2 (Rankin) professional meetings (Phycological Society of America annual meeting, Northeast Algal Society meeting) during the project period.

Outreach Activities:

Two types of outreach activities occurred during the project. First, the information on the bacterial community was shared with the Joint Genome Institute (DoE). Second, Brawley creates a service learning opportunity for undergraduates in her SMS 372 class each spring (Marine and Freshwater Algae). As a result of the research activities of this proposal, the one devised in spring 2012 concerned the stress tolerances and identity of Porphyra spp. sensu lato in bags of 'Porphyra umbilicalis' sold by a local Maine company, nationally and internationally. The students (7 undergraduates, 1 grad student) determined that 2 of the 3 bags subsampled contained significant amounts of a related genus (Wildemannia birdie) as well as Porphyra umbilicalis. The product was bought at a local grocery store, sequenced at two chloroplast genes, and examined anatomically with sections of thalli in brightfield microscopy to search for reproductive structures. Students prepared a Power Point on their research and presented the results to the company. The company changed its labeling on product for sale as a result.

Journal Publications

Miranda, LM; Grossman, A; Brawley, SH, "BACTERIAL COMMUNITY STRUCTURE IN PORPHYRA UMBILICALIS FROM THE MAINE SHORE IN AUTUMN AND WINTER ASSESSED THROUGH 16S RDNA SEQUENCING", JOURNAL OF PHYCOLOGY, p. S36, vol. 47, (2011). Published,

Miranda LN; Hutchison K; Grossman AR; Brawley SH, "Diversity and abundance of the bacterial community of the red macroalga Porphyra umbilicalis: Did bacterial farmers produce macroalgae?", PLoS One, p. , vol. , (2013). Submitted,

Miranda L; Hutchison K; Grossman AR; Brawley SH, "Bacterial communities associated with blades of the red macroalga Porphrya umibilicalis (???laver???)", American Society of Cell Biology Abstracts, abstract 3065, p. , vol. , (2012). Published,

Rankin JA; Manning J; Miranda LN; Brawley SH, "Low salinity tolerance of Porphyra umbilicalis", J. Phycol., p. , vol. S44, (2012). Published,

York GE; Arnold DS; Brehm LJ; DeMerchant JR; Jensen AJ; Lyczkowski ER; Ouellette AR; Rankin JA; Phillips JC; Brawley SH; Erhart S, "Identification of Porphyra species in Maine Coast Sea Vegetables ??????Laver?????? using molecular techniques.", Journal of Phycology, p. , vol. S22, (2012). Published,

Books or Other One-time Publications

Web/Internet Site

Other Specific Products

Contributions

Contributions within Discipline:

The metagenomic information on microbial communities associated with P.um.1 seems likely to be essential in completion of the Porphyra umbilicalis genome project. This alga is commercially important and long-considered a key target for better understanding algal evolution and metabolism (e.g. stress tolerance).

Contributions to Other Disciplines:

The pyrosequencing study completed of the bacterial metagenome of Porphyra umbilicalis makes a major contribution to understanding interactions between bacteria and macroalgae. It is one of the first such studies of any macroalga. Scientists interested in symbiosis will also benefit from this work.

Contributions to Human Resource Development:

Dr. Lilibeth Miranda is a talented molecular biologist who received training in cell biology and molecular biology on a new system to her. She is now adept at algal culture, bright-field and fluorescence microscopy, cell isolation, electroporation, construction of plasmids for transformation studies. This background was key to her recent hire by the National University of Singapore to do research and manage a harmful algal bureau.

Mr. Joseph Rankin and Mr. Joshua Manning are seniors at the University of Maine who participated in the project. Mr. Rankin completed his required senior capstone project while working on and supporting the EAGER research. Both students made individual presentations of their research to national professional audiences at scientific meetings. Mr. Manning received a NSF REU at UT's Marine Science Institute last summer in part based upon his experience on the EAGER research grant.

Contributions to Resources for Research and Education:

I reported briefly on the service learning research project, done by my undergraduate Marine & Freshwater Algae class in spring 2012, which gave students both basic and applied research experience, beginning with framing their own hypotheses, learning good experimental design, and a combination of intensive sequencing and microscope work.

UMaine Today, the University of Maine's public magazine, also ran an article in Summer 2012 about my Porphyra research. This magazine is distributed widely to the public (libraries, legislators, online, etc.).

Contributions Beyond Science and Engineering:

The service learning project led to a keener appreciation in a major Maine company of the need to apply scientific methodology to harvest techniques, and changed their labeling information at their online company site and on product sold commercially.

Conference Proceedings

Categories for which nothing is reported:

Organizational Partners

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